

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 14 November 2000 (14.11.00)	
International application No. PCT/GB00/01029	Applicant's or agent's file reference 88.96,68343/001
International filing date (day/month/year) 20 March 2000 (20.03.00)	Priority date (day/month/year) 19 March 1999 (19.03.99)
Applicant HAGERLID, Peter et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 17 October 2000 (17.10.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Olivia TEFY Telephone No.: (41-22) 338.83.38
---	---

PATENT COOPERATION TREATY

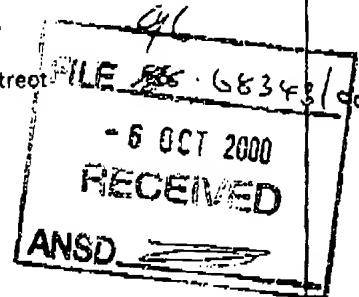
PCT

NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

SAMUELS, Adrian, J.
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
ROYAUME-UNI

Date of mailing (day/month/year) 28 September 2000 (28.09.00)		IMPORTANT NOTICE	
Applicant's or agent's file reference 88.96.68343/001			
International application No. PCT/GB00/01029	International filing date (day/month/year) 20 March 2000 (20.03.00)	Priority date (day/month/year) 19 March 1999 (19.03.99)	
Applicant PYROSEQUENCING AB et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AG, AU, DZ, KP, KR, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
AE, AL, AM, AP, AT, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EA, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, OA, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).
3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 28 September 2000 (28.09.00) under No. WO 00/58455

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF
THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 28 September 2000 (28.09.00)	IMPORTANT NOTICE
Applicant's or agent's file reference 88.96.68343/001	International application No. PCT/GB00/01029
<p>The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.</p>	

3537626

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 88.96,68343/001	FOR FURTHER ACTION <small>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</small>	
International application No. PCT/GB 00/ 01029	International filing date (day/month/year) 20/03/2000	(Earliest) Priority Date (day/month/year) 19/03/1999
Applicant PYROSEQUENCING AB et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

PRESSURE-PULSE ACTUATED LIQUID DISPENSING APPARATUS

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/02

//B01J19/00,C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01L G01N B01J C12Q C12M B05C B67D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A A	US 5 435 462 A (FUJII HIDEYO) 25 July 1995 (1995-07-25) column 1, line 46 -column 4, line 4 figure 1 --- WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14) page 13, line 15 -page 13, line 18 page 51, line 1 -page 51, line 12 page 52, line 12 -page 54, line 11 figure 2 --- -/--	1-3,19, 20,23 17,18 1-4,17, 23

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 June 2000

Date of mailing of the international search report

18.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV RijswijkTel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Koch, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 98 29736 A (GENOMETRIX INC) 9 July 1998 (1998-07-09) page 10, line 6 -page 10, line 21 page 15, line 10 -page 15, line 14 page 21, line 6 -page 21, line 10 page 22, line 12 -page 22, line 27 page 23, line 16 -page 24, line 21 page 27, line 10 -page 27, line 28 page 42, line 21 -page 43, line 8 page 44, line 12 -page 44, line 20 page 61, line 20 -page 62, line 11 figure 4</p> <p>-----</p>	1,4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet(s)

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4, 17-20, 23

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4,17-20,23

Cassette with plurality of cartridges for dispensing liquid therefrom, the cartridge being closed at one end and having an outlet port from which liquid is ejected by a pulse of pressurised gas.

2. Claims: 5-16,21,22

Liquid dispensing apparatus with liquid reservoir, outlet port and driving means for generating a pulse of gas in order to force the liquid out of the outlet port.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01029

Patent document cited in search report		Application date	Patent family member(s)	Publication date
US 5435462	A	25-07-1995	NONE	

WO 9820020	A	14-05-1998	US 6024925 A	15-02-2000
			AU 5106998 A	29-05-1998
			AU 5198098 A	29-05-1998
			DE 19782095 T	23-03-2000
			DE 19782096 T	23-03-2000
			EP 0954612 A	10-11-1999
			EP 0937096 A	25-08-1999
			NO 992168 A	06-07-1999
			NO 992169 A	06-07-1999
			WO 9820166 A	14-05-1998
			AU 5247298 A	29-05-1998
			DE 19782097 T	14-10-1999
			EP 0937097 A	25-08-1999
			NO 992167 A	05-07-1999
			WO 9820019 A	14-05-1998

WO 9829736	A	09-07-1998	AU 6646398 A	31-07-1998
			EP 0990142 A	05-04-2000

19 JUN 2001

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

161

Applicant's or agent's file reference 98.68343/001		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB00/01029	International filing date (day/month/year) 20/03/2000	Priority date (day/month/year) 19/03/1999	
International Patent Classification (IPC) or national classification and IPC B01L3/02			
Applicant PYROSEQUENCING AB et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 17/10/2000	Date of completion of this report 15.06.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Sembritzki, T Telephone No. +49 89 2399 8626



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01029

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
- Description, pages:**

1-12 as originally filed

Claims, No.:

8 (part), 9-23 as originally filed

1-7, 8 (part) as received on

25/04/2001 with letter of

25/04/2001

Drawings, sheets:

1/2, 2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer-readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01029

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-16, 21, 22.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 5-16, 21, 22.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01029

1. Statement

Novelty (N)	Yes:	Claims	1-4, 18, 20, 23
	No:	Claims	17, 19
Inventive step (IS)	Yes:	Claims	1-4, 23
	No:	Claims	18, 20
Industrial applicability (IA)	Yes:	Claims	1-4, 17-20, 23
	No:	Claims	

2. Citations and explanations **see separate sheet**

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01029

Reference is made to the following documents:

- D1: US-A-5 435 462 (FUJII HIDEYO) 25 July 1995 (1995-07-25)
D2: EP 0941854 A

Point V:

1. Novelty and Inventive Step

- 1.1 The definition of claim 1 is in several points unclear (see **Point VIII** below). However, none of the documents cited in the search report discloses or suggests a cassette comprising a plurality of cartridges, wherein each cartridge is adapted to dispense drops of 10 - 500 nl by applying pressure pulses to the liquid contained in the cartridges. Accordingly, the subject-matter of claim 1 meets the requirements of Articles 33(2) and (3) PCT.
- 1.2 Claims 2-4 are dependent on claim 1 and as such also meet the requirements of the PCT with respect to novelty and inventive step.
- 1.3 Independent claims 17 and 19 neither define a pulse of pressurised gas or any adaptation thereto nor a specified droplet volume. Accordingly, both claims define nothing else but a usual commercially available ink-jet printer cartridge or respectively a cassette comprising these cartridges. Consequently, the subject-matter of claims 17 and 19 is not novel (Article 33(2) PCT).
- 1.4 The subject-matter of the other dependent claims seems to contain only features which are either known from the prior art and which a skilled person could easily combine without any inventive skill, or which are the result of a normal design procedure followed by a skilled person (Article 33(3) PCT). From D1 it is for example known, that the liquid reservoir contains a quantity of liquid and is closed at one end thereof by a frangible sealing means (claim 18) (see D1, figure 1). Cartridges with a plurality of integrally formed reservoirs and protruding nozzles (claims 17) are for example known from every ink-jet printer.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01029

2. Industrial application

The industrial applicability is obvious.

Point VII:

3. Certain defects

- 3.1 The independent claims are not in the two-part form in accordance with Rule 6.3(b) PCT, which in the present case would be appropriate.
- 3.2 The features of the claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

Point VIII:

4. Clarity

- 4.1 In view of the description and the figures it is obvious, that in independent claim 1 essential features are missing (Article 6 PCT):

- It is not clearly specified, that each pulse of gas creates only one single droplet of a volume in the range of 10-500 nl. In a usual perfume vaporizer or atomizer one pressure pulse generated by pressing the vaporizer button creates a plurality of fine droplets.

- Furthermore claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The problem in the present case does not seem to be that drops are formed, but how the single droplet of the well defined small size are created. However, the claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01029

- 4.2 Independent claims 17 and 19 neither contain any information about a pulse of pressurised gas nor any feature which could be interpreted as an adaptation to this technique. Both claims are therefore on the one hand not supported by the description and do not contain all the features which are necessary to carry out the technique mentioned in the description (Article 6 PCT), on the other hand independent claims 1, 17 and 19 formally lack unity (Rule 13.1 PCT) since they are not so linked as form a single inventive concept.

- 13 -

1. A cassette comprising a plurality of cartridges for dispensing drops of liquid in the range 10 - 500 nl therefrom, each cartridge comprising a liquid reservoir and an outlet member mounted so as to close an opening in the liquid reservoir at one end thereof, said outlet member providing an outlet port through which said liquid can be forced by a pulse of pressurised gas applied to the liquid at the other end of the liquid reservoir, thereby forming a said drop.
2. A cassette as claimed in claim 1 wherein at least the liquid reservoirs of said cartridges are formed integrally with one another.
3. A cassette as claimed in claim 1 or 2 wherein said outlet port comprises an aperture in said outlet member.
4. A cassette as claimed in claim 1 or 2 wherein said outlet port comprises a protruding nozzle.
5. A liquid dispensing apparatus comprising a liquid reservoir, an outlet port and driving means for forcing liquid through said outlet port, said driving means comprising means for generating a pulse of gas which impinges upon liquid in the reservoir so as to force liquid through the outlet port.
6. An apparatus as claimed in claim 5, wherein said outlet port is formed integrally with the or each liquid reservoir.
7. An apparatus as claimed in claim 5 wherein said outlet port is provided by a separate outlet member which is mounted so as to close an opening in the liquid reservoir.

- 13a -

8. An apparatus as claimed in claims 5, 6 or 7 wherein said outlet port and liquid reservoir are moulded from

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 September 2000 (28.09.2000)

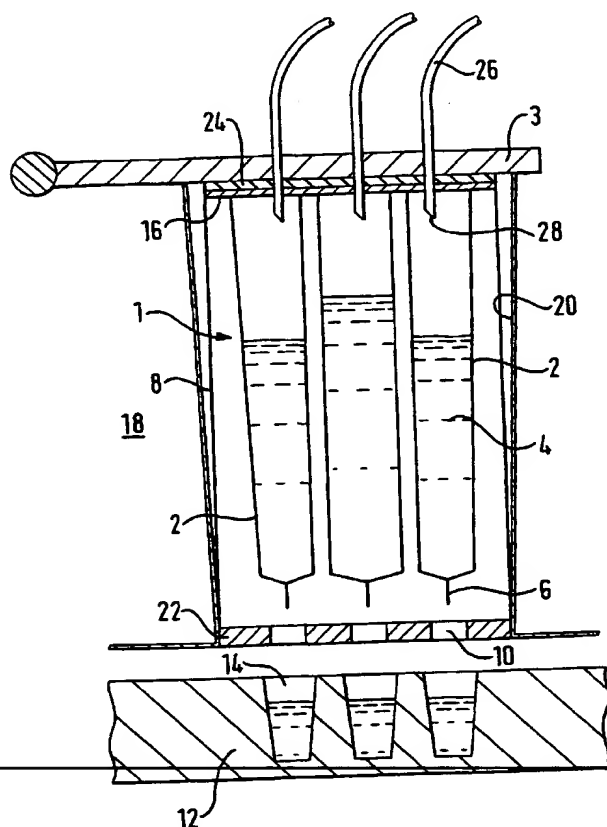
PCT

(10) International Publication Number
WO 00/56455 A3

- (51) International Patent Classification⁷: **B01L 3/02**
// B01J 19/00, C12Q 1/68
- (21) International Application Number: **PCT/GB00/01029**
- (22) International Filing Date: **20 March 2000 (20.03.2000)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
9906477.6 **19 March 1999 (19.03.1999)** **GB**
- (71) Applicant (for all designated States except US): **PY-ROSEQUENCING AB [SE/SE]**; Vallongatan 1, S-752 28 Uppsala (SE).
- (71) Applicant (for GB only): **PIESOLD, Alexander, James** [GB/GB]; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): **HAGERLID, Peter** [SE/SE]; Sköldungagatan 1, S-753 34 Uppsala (SE). **EHRING, Hanno** [DE/SE]; Karlsrogatan 83a, S-752 39 Uppsala (SE). **EKSTRÖM, Björn** [SE/SE]; Järpvägen 27, S-756 53 Uppsala (SE).
- (74) Agent: **SAMUELS, Adrian, J.**; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.**
- (84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent**

[Continued on next page]

(54) Title: **PRESSURE-PULSE ACTUATED LIQUID DISPENSING APPARATUS**



(57) Abstract: A liquid dispensing apparatus comprises a liquid reservoir (32), an outlet port (6; 36) and driving means for forcing liquid (4) through the outlet port (6; 36). The driving means generates a pulse of gas which impinges upon the liquid (4) in the reservoir (32) so as to force liquid (4) through the outlet port (6; 36). Also disclosed is a cassette comprising a plurality of cartridges for dispensing liquid therefrom. Each cartridge comprises a liquid reservoir (32) and an outlet member (36) mounted so as to close an opening (34) in the liquid reservoir (32). The outlet member (36) provides an outlet port through which the liquid can be forced by a pulse of pressurised gas.

WO 00/56455 A3



(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

(88) Date of publication of the international search report:
25 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/ 00/01029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/02

//B01J19/00,C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L G01N B01J C12Q C12M B05C B67D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 435 462 A (FUJII HIDEYO) 25 July 1995 (1995-07-25) column 1, line 46 -column 4, line 4 figure 1	1-3,19, 20,23 17,18
A	---	
A	WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14) page 13, line 15 -page 13, line 18 page 51, line 1 -page 51, line 12 page 52, line 12 -page 54, line 11 figure 2 --- -/--	1-4,17, 23

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

8 June 2000

Date of mailing of the international search report

18.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Koch, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/01029

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
------------	--	-----------------------

A

WO 98 29736 A (GENOMETRIX INC)
9 July 1998 (1998-07-09)
page 10, line 6 -page 10, line 21
page 15, line 10 -page 15, line 14
page 21, line 6 -page 21, line 10
page 22, line 12 -page 22, line 27
page 23, line 16 -page 24, line 21
page 27, line 10 -page 27, line 28
page 42, line 21 -page 43, line 8
page 44, line 12 -page 44, line 20
page 61, line 20 -page 62, line 11
figure 4

1,4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet(s)

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 17-20, 23

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4,17-20,23

Cassette with plurality of cartridges for dispensing liquid therefrom, the cartridge being closed at one end and having an outlet port from which liquid is ejected by a pulse of pressurised gas.

2. Claims: 5-16,21,22

Liquid dispensing apparatus with liquid reservoir, outlet port and driving means for generating a pulse of gas in order to force the liquid out of the outlet port.

INTERNATIONAL SEARCH REPORT

International patent family members

International Application No

PC 00/01029

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5435462	A	25-07-1995	NONE	
WO 9820020	A	14-05-1998	US 6024925 A	15-02-2000
			AU 5106998 A	29-05-1998
			AU 5198098 A	29-05-1998
			DE 19782095 T	23-03-2000
			DE 19782096 T	23-03-2000
			EP 0954612 A	10-11-1999
			EP 0937096 A	25-08-1999
			NO 992168 A	06-07-1999
			NO 992169 A	06-07-1999
			WO 9820166 A	14-05-1998
			AU 5247298 A	29-05-1998
			DE 19782097 T	14-10-1999
			EP 0937097 A	25-08-1999
			NO 992167 A	05-07-1999
			WO 9820019 A	14-05-1998
WO 9829736	A	09-07-1998	AU 6646398 A	31-07-1998
			EP 0990142 A	05-04-2000



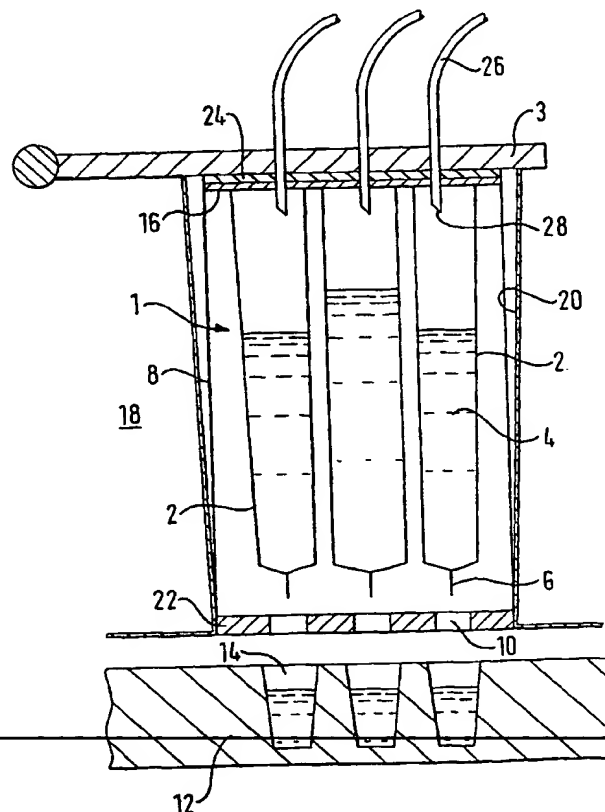
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : B01L	A2	(11) International Publication Number: WO 00/56455
		(43) International Publication Date: 28 September 2000 (28.09.00)
<p>(21) International Application Number: PCT/GB00/01029</p> <p>(22) International Filing Date: 20 March 2000 (20.03.00)</p> <p>(30) Priority Data: 9906477.6 19 March 1999 (19.03.99) GB</p> <p>(71) Applicant (for all designated States except US): PYROSEQUENCING AB [SE/SE]; Vallongatan 1, S-752 28 Uppsala (SE).</p> <p>(71) Applicant (for GB only): PIESOLD, Alexander, James [GB/GB]; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): HAGERLID, Peter [SE/SE]; Sköldungagatan 1, S-753 34 Uppsala (SE). EHRING, Hanno [DE/SE]; Karlsrogatan 83a, S-752 39 Uppsala (SE). EKSTRÖM, Björn [SE/SE]; Järpvägen 27, S-756 53 Uppsala (SE).</p> <p>(74) Agent: SAMUELS, Adrian, J.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).</p>		<p>(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>

(54) Title: LIQUID DISPENSING APPARATUS

(57) Abstract

A liquid dispensing apparatus comprises a liquid reservoir (32), an outlet port (6; 36) and driving means for forcing liquid (4) through the outlet port (6; 36). The driving means generates a pulse of gas which impinges upon the liquid (4) in the reservoir (32) so as to force liquid (4) through the outlet port (6; 36). Also disclosed is a cassette comprising a plurality of cartridges for dispensing liquid therefrom. Each cartridge comprises a liquid reservoir (32) and an outlet member (36) mounted so as to close an opening (34) in the liquid reservoir (32). The outlet member (36) provides an outlet port through which the liquid can be forced by a pulse of pressurised gas.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Liquid Dispensing Apparatus

5 This invention relates to liquid dispensing apparatus and in particular to liquid dispensing apparatus which are able to dispense small volumes of liquid.

10 In certain applications it is desirable to be able to dispense very small volumes of liquid from a container, for example in the range 10-500 nanolitres (nl). Drops of this size cannot normally be produced by pumping liquid out of a capillary since surface tension will hold the drop to the tip of the capillary until it is sufficiently large that its weight overcomes this
15 surface tension. This does not happen until the volume of the drop is of the order of 10 to 50 microlitres - i.e. 2 to 3 orders of magnitude greater than the range of interest.

20 Devices are available which overcome this limitation by forcing a measured amount of liquid through the tip of a nozzle using a piezo-electric actuator acting on the liquid. However such systems are expensive and may only be used with a single type of liquid at a time - thus multiplying the cost where
25 several different liquids need to be dispensed.

It is an object of the present invention to provide an improved apparatus and when viewed from a first aspect the invention provides a liquid dispensing apparatus comprising a liquid reservoir, an outlet port
30 and driving means for forcing liquid through said outlet port, said driving means comprising means for generating a pulse of gas which impinges upon liquid in the reservoir so as to force liquid through the outlet port.

35 Thus it will be seen that in accordance with the invention liquid may be dispensed by forcing it through the outlet port by means of a pulse of gas. The volume of liquid dispensed may be controlled by adjusting the

- 2 -

amplitude and duration of the gas pulse. It has been found that this enables accurate control of the volume dispersed, down to the order of 50-500 nl in the preferred embodiment. Furthermore as a gas is used as the medium for transmitting force to the liquid, it is not necessary for any part of the driving means to be in contact with the liquid to be dispensed. This has clear advantages both from the point of view of preventing contamination of the liquid and also the reduced necessity to clean the apparatus.

As the driving impulse is applied to liquid in the reservoir, it is not necessary to have any part of the driving means arranged in the vicinity of the outlet port. This means that the outlet port can be simple to manufacture and the outlet port may even be provided on a disposable member.

In one preferred arrangement in accordance with the invention, the outlet port is an integral part of the apparatus. In a particularly preferred embodiment one or both of the outlet port and the liquid reservoir are moulded from a suitable plastics material, thereby allowing them to be made disposable. More preferably the outlet port and liquid reservoir are both moulded from suitable plastics, most preferably integrally with each other, to form a cartridge. Alternatively such a cartridge could be made from one or more other suitable materials such as silica or stainless steel.

Cartridges of the kind described above may be provided singly, but preferably a plurality are provided in a cassette. Such an arrangement is novel and advantageous in itself and thus when viewed from a second aspect the present invention provides a cassette comprising a plurality of cartridges for dispensing liquid therefrom, each cartridge comprising an outlet port in the form of a protruding nozzle and a liquid reservoir integrally formed therewith.

Preferably the cartridges are formed integrally

- 3 -

with one another to form said cassette.

5 The invention makes it possible, at least in some preferred embodiments, to provide cartridges pre-filled with the necessary liquid and having the required outlet
10 port formed integrally therewith. All that is necessary then is simply to place the liquid reservoir of the cartridge or each cartridge of a cassette of cartridges in gaseous communication with a means to generate gas pulses, in order to form a liquid dispensing apparatus
15 in accordance with the first aspect of the invention. This is extremely convenient to a user who thus does not need to be concerned with filling reservoirs with liquid, cleaning the outlet port etc. Indeed in at least preferred embodiments of this aspect of the
20 invention, contact with the liquid to be dispensed may be completely avoided.

In other preferred arrangements the outlet port is provided by a separate outlet member which is mounted so as to close an opening in the liquid reservoir. This is
25 advantageous since it allows the liquid reservoir and outlet member to be made from different materials each of which can be optimised for its function. For example, the dimensions of the liquid reservoir may be relatively uncritical and so it could be made relatively
30 inexpensively, e.g. as an injection moulded plastics component. On the other hand, the dimensions of the outlet port of the outlet member are likely to be more critical and it might for example be made to a tighter tolerance, e.g. from metal.

35 As above, the outlet member and liquid reservoir preferably together form a cartridge, and in preferred embodiments a cassette comprising a plurality of such cartridges is provided. Thus when viewed from a further aspect the present invention provides a cassette
40 comprising a plurality of cartridges for dispensing liquid therefrom, each cartridge comprising a liquid reservoir and an outlet member mounted so as to close an

- 4 -

opening in the liquid reservoir, said outlet member providing an outlet port through which said liquid can be forced by a pulse of pressurised gas.

Preferably at least the liquid reservoirs of said
5 cartridges are formed integrally with one another to form said cassette.

In some preferred embodiments the outlet port comprises a protruding nozzle, e.g. a capillary tube. The bore of such a nozzle is preferably within the range
10 0.05 to 0.2 mm, most preferably about 0.1 mm. The length of the nozzle is preferably in the range 1 to 15 mm, most preferably 5 mm.

More preferably, the outlet port comprises an aperture in a wall of the liquid reservoir. The
15 aperture is chosen in size to give the required volume of drop when a given pressure pulse is applied. The aperture however should be small enough that the surface tension in the liquid in the reservoir prevents it from leaking through.

In accordance with the first and second aspects of the invention, the wall of the liquid reservoir in which the aperture is provided may be integral with the reservoir but preferably it is a separate wall member mounted so as to close an opening in the liquid
20 reservoir.

Where, as is preferred, a plurality of cartridges together form a cassette, each may have its own such wall member or two or more of the cartridges may share a wall member with an aperture for each cartridge.

30 A single aperture may be provided for the or each liquid reservoir, or more may be provided to increase the volume that may be dispensed without increasing the size of the aperture (which could give rise to a greater probability of leaking).

35 The aperture could be any shape, but most conveniently it is round. However, if larger volumes of liquid are required an elongate slit could for example

by employed.

In preferred embodiments the aperture is between 2 and 300 micrometers wide, preferably approximately 40 micrometers. If the aperture is a slit it could for example be 40 x 500 micrometers.

The wall member could be made from any suitable material, e.g. plastics, metal, glass or ceramics. Preferably it has the opposite relationship to water as the liquid for which it is intended to be used, i.e. preferably the member is hydrophilic if the liquid is hydrophobic and vice versa. It may have any suitable thickness, but preferably it is between 10 micrometers and 1000 micrometers (1 mm), most preferably approximately 50 micrometers.

The aperture may be formed by suitably precise technique e.g. etching, electroplating, laser drilling or mechanical drilling.

The wall member may be fixed to the or each liquid reservoir in any suitable manner e.g. gluing, ultrasonic welding, friction welding etc. In one preferred embodiment the liquid reservoir tapers towards its open end and the wall member is pressed into an interference fit with the tapered end. The wall member therefore preferably has the same peripheral shape as the liquid reservoir, most preferably this being round. The diameter of the wall member in such a case is preferably between 0.5 and 6 mm, most preferably approximately 2 mm.

The cartridge or each cartridge of a cassette may be filled with a suitable liquid prior to use, e.g. a lyophilised reagent which is dissolved in water when required. The preferred embodiments of the invention in which the liquid reservoir is adjacent the outlet port are advantageous in this context since just the required amount of reagent can be made up as required without extra being required to accommodate dead space in pipes etc.

- 6 -

Cassettes of cartridges in accordance with the invention as described above are useful in many applications including but not limited to immuno assays, cell assay, drug screening and they are particularly useful for sequencing DNA using the method often referred to as "Sequencing By Synthesis" as they allow for example a cassette having cartridges containing each of the four nucleotides required for DNA sequencing. Preferably therefore the cartridges respectively contain one or more nucleotides. The ability to provide a plurality of cartridges in a single cassette further allows a complete set of reagents required for a sequencing programme to be provided. Thus preferably further cartridges of the cassette respectively contain one or more enzymes, more preferably selected from a group comprising polymerase, luciferase, adenosine triphosphate (ATP) sulfurylase, and a nucleotide-degrading enzyme such as apyrase. Such a set of reagents is particularly useful for the method of genetic sequencing set out in WO 98/13523. Indeed it will be appreciated that the invention extends to a method of genetic sequencing using a liquid dispensing apparatus or cassette as herein described.

As discussed above, cartridges of the kind described above may be filled by a user with the appropriate liquid as required. Alternatively the cartridge or each cartridge of a cassette is pre-filled with the appropriate liquid and sealed, the seal being broken upon mounting the cartridge into an apparatus which includes the gas pulse generation means. Preferably the seal is broken by the action of mounting the cartridge into the apparatus. Advantageously a conduit for establishing gaseous communication with the interior of the liquid reservoir of the cartridge breaks the seal.

It will be seen from the above that a sealed cartridge containing a liquid to be dispensed by means

of apparatus in accordance with the first aspect of the invention is in itself not only novel, but particularly convenient for a user and thus when viewed from a further aspect the present invention provides a
5 cartridge comprising a liquid reservoir having a predetermined liquid received therein, said reservoir being closed at one end thereof by frangible sealing means, and an outlet port attached to or integrally formed with said reservoir and in fluid communication
10 therewith.

As an alternative it will be seen that the invention extends to a cartridge for use in a liquid dispensing apparatus as hereinbefore described comprising a liquid reservoir, and an outlet port
15 attached to or integrally formed with said reservoir and in fluid communication therewith, in combination with a reagent, preferably a nucleotide or enzyme.

The reagent e.g. nucleotide or enzyme could be in a suitable liquid form or could be lyophilised. In the
20 latter case a readily available diluent such as water could be used, or a suitable diluent could instead also be provided as part of the combination.

The frangible sealing means preferably comprises a foil membrane e.g. of aluminium covering an opening onto
25 the reservoir. This is a cost-effective way of retaining liquid in the reservoir without it becoming contaminated, whilst at the same time being relatively easy to pierce - e.g. by a gas nozzle associated with the gas pulse generation means.

30 The predetermined liquid preferably comprises a nucleotide or an enzyme.

Where an enzyme is provided, either as the predetermined liquid in a pre-filled cartridge or as a separate reagent to be added by a user, the enzyme is
35 preferably selected from a group comprising polymerase, luciferase, adenosine triphosphate (ATP) sulfurylase, and a nucleotide-degrading enzyme such as apyrase.

- 8 -

The means for generating the gas pulses may comprise any suitable means - e.g. a bellows arranged to undergo a rapid reduction in volume. Preferably however the pulse generation means comprises a source of pressurised gas which may be selectively placed into communication with the liquid reservoir. Such arrangements are especially beneficial since a single source of pressurised gas may be used to drive a plurality of liquid reservoirs, thereby giving a significant saving in cost over arrangements where driving means are individually provided for each of a number of nozzles. Means for selectively placing the liquid reservoir into communication with the source of gas may be provided for each of such liquid reservoirs or two or more of them may be associated with a single selection means so that their contents are dispensed simultaneously.

Preferably one or more valves is provided to effect said selective communication and in the preferred embodiment an electromagnetic valve is used. This is particularly advantageous since electromagnetic valves can be operated very quickly and accurately with little mechanical wear.

Where, as is preferred, the apparatus for dispensing liquid comprises gas pulse generation means in the form of means for selectively communicating a source of pressurised gas with the liquid reservoir, the source of pressurised gas may be comprised within the apparatus. Most preferably the source of pressurised gas comprises a compressor supplying a pressure reservoir. Alternatively, the apparatus may comprise a gas inlet for connection to an external source of pressurised gas. In either case the apparatus of the invention preferably comprises means to regulate the pressure of the incoming gas thereby allowing the amplitude of the pulses generated to be accurately controlled and thus the volume of liquid to be

accurately controlled as a consequence. The pressure regulation means may comprise a mechanical release valve or the like. Preferably however such means comprises an electronic pressure sensor which is used to monitor the pressure in the reservoir or gas inlet and means for increasing or decreasing said pressure in response to the signal from the pressure sensor to maintain the pressure within a predetermined range.

The gas pulse generation means preferably generates pressure pulses having an amplitude in the range 200 to 1000 millibars, with a pulse width in the range 1-1000 milliseconds. This enables volumes in the range 50 nl to 10000 nl (10 μ l) to be dispensed.

Certain preferred embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings in which:-

Figure 1 is a schematic view of a cassette of cartridges and liquid dispensing apparatus in accordance with the invention; and

Figure 2 is a cross-section through a cartridge in accordance with a second embodiment of the invention.

Turning to Figure 1 there may be seen a liquid dispensing apparatus comprising a cartridge cassette designated generally by the reference numeral 1. This cassette contains eight cartridges 2, three of which are visible in the drawing. Each cartridge 2 is partly filled with a reagent 4 used for genetic sequencing. The volume of the cartridges is approximately 2 ml. Different reagents 4 are provided in each cartridge 2. Specifically, four of the cartridges 2 contain the deoxynucleotide triphosphate of one each of the four bases cytosine, guanine, adenosine and thymine. Four further cartridges 2 contain polymerase, luciferase, apyrase and ATP sulfurylase. Equally however some or all of the cartridges could have the same contents.

At the end of each cartridge 2 there is a capillary nozzle 6. This nozzle has a bore of 0.1 mm. In the

- 10 -

depicted embodiment the nozzle 6 is integrally moulded with the rest of the cartridge 2 from a polymer. However the cartridge could be formed with means to receive a separate nozzle.

5 The cassette 1 has an outer case 8 which protects the cartridges 2 from contamination by the user handling them and conversely protects the user from having to come into contact with the liquid being dispensed or the very fine, i.e. sharp nozzles 6. Apertures 10 are
10 provided in the base of the cassette casing 8 which are aligned with the nozzles 6 to allow the liquid through.

 At the upper end of the cassette 1 a thin metal foil 16 is provided over the tops of the cartridges 2 to seal them. The foil 16 extends across the open upper
15 end of the cassette 1 formed by the side walls of the casing 8, but slightly downwardly set from the top edge so as to leave an upstanding lip around the top of the foil 16.

 The cassette 1 is installed in a carriage 18 which
20 can be moved laterally in both directions over a Micro Titre Plate (MTP) 12. The MTP has 96 wells 14, three of which are shown. The cassette 1 is actually received in a downwardly tapering chamber 20, so as to rest on an apertured plate 22, the apertures of which are in
25 alignment with the apertures 10 in the cassette and, when the carriage 18 is properly positioned, also with the wells 14 of the MTP 12.

 The cassette 1 is retained in the chamber 20 by means of a hinged lid 3, having a rubber seal 24 on its
30 underside which is a tight fit inside the lip at the top edge of cassette casing 8. The seal 24 is arranged also to ensure that the cartridges 2 are sealed with respect to one another as well as with respect to the lid 3.

 Mounted in the lid 3 is an array of punching
35 cannulae 28, one for each of the cartridges 2. These cannulae 28 have sharp tips and so as the lid 3 is hinged downwardly onto the cassette 1 in the chamber 20,

- 11 -

the cannulae 28 pierce the foil seal 28. The other ends of the cannulae are each connected by means of a pipe 26 to an electromagnetic valve (not shown) which selectively connects the pipe 26 to a source of
5 pressurised gas for a predetermined time to produce a pulse of gas. The pressurised gas is generated by a compressor type EC Genius/M 202 2305 EU.SV.C which is commercially available from Fini, Zola, Predosa, Bo - Italy.

10 The pulse of gas forces a measured amount of reagent 4 out of the cartridge 2, through the nozzle 6 and into the corresponding well 14 on the MTP 12. A 500 millibar pulse, for 10 milliseconds dispenses approximately 200 nanolitres of reagent into the well
15 14. Reagent is only dispensed from one of the cartridges 2 at a time although simultaneous dispensing is also possible and this could be useful e.g. where a plate having a greater number of wells was used with an interleaved series of wells used so that dispensing into
20 the wells of several distinct series can be carried out without moving the carriage 18.

Once the reagents in the cartridges have been used, the cassette may simply be removed by opening the lid 3 and replaced with a new one, the fresh seals being
25 broken by the cannulae 28. Since neither the cannulae 28 nor any other permanent part of the apparatus comes into contact with the reagents at any stage, there is little chance of contamination and it is not necessary to clean the apparatus between uses, even if different
30 reagents are being used.

Figure 2 shows part of the cartridge 30 of another embodiment of the invention. It will be seen that the liquid reservoir 32 tapers at its lower end 32a to form an opening circumscribed by an annular lip 34. The
35 reservoir 32 and lip 34 are integrally formed in injection moulded plastics. The bottom opening is closed by a thin metallic foil disc 36 which has a central

- 12 -

aperture drilled into it. The disc 36 is pressed into the end of the liquid reservoir 32 thereby forming a fluid-tight interference fit. The disc is 2 mm in diameter and 50 micrometers thick. The aperture is
5 essentially circular with a diameter of approximately 40 micrometers. This is sufficiently small to prevent any leakage of liquid from the reservoir 32.

This embodiment operates in exactly the same way as the embodiment already described, although it will be
10 appreciated that it has the additional benefits that the size of the aperture is easier to control precisely than is the bore of the capillary tube, and furthermore there is no potentially hazardous sharp tip.

Although embodiments of the invention having a
15 cassette comprising several cartridges have been described, it will be understood by those skilled in the art that the invention is not limited to using several cartridges and that a single cartridge reservoir could be used instead. Furthermore, the liquid reservoir need
20 not be removable and could instead be an integral part of the apparatus.

Claims:

1. A cassette comprising a plurality of cartridges for dispensing liquid therefrom, each cartridge comprising a liquid reservoir and an outlet member mounted so as to close an opening in the liquid reservoir, said outlet member providing an outlet port through which said liquid can be forced by a pulse of pressurised gas.
 2. A cassette as claimed in claim 1 wherein at least the liquid reservoirs of said cartridges are formed integrally with one another.
 3. A cassette as claimed in claim 1 or 2 wherein said outlet port comprises an aperture in said outlet member.
 4. A cassette as claimed in claim 1 or 2 wherein said outlet port comprises a protruding nozzle.
 5. A liquid dispensing apparatus comprising a liquid reservoir, an outlet port and driving means for forcing liquid through said outlet port, said driving means comprising means for generating a pulse of gas which impinges upon liquid in the reservoir so as to force liquid through the outlet port.
 6. An apparatus as claimed in claim 5, wherein said outlet port is formed integrally with the or each liquid reservoir.
 7. An apparatus as claimed in claim 5 wherein said outlet port is provided by a separate outlet member which is mounted so as to close an opening in the liquid reservoir.
 8. An apparatus as claimed in claims 5, 6 or 7 wherein said outlet port and liquid reservoir are moulded from
-

plastics to form a cartridge.

5 9. An apparatus as claimed in claim 8 comprising a cassette, said cassette comprising a plurality of said cartridges.

10 10. An apparatus as claimed in any of claims 5 to 9 wherein the pulse generation means comprises a source of pressurised gas which may be selectively placed into communication with the liquid reservoir.

15 11. An apparatus as claimed in claim 10 wherein one or more electromagnetic valves is provided to effect said selective communication.

20 12. An apparatus as claimed in any of claims 5 to 11 wherein said gas pulse generation means is arranged to generate pulses having an amplitude in the range 200 to 1000 millibars.

25 13. An apparatus as claimed in any of claims 5 to 12 wherein said gas pulse generation means is arranged to generate pulses having a width in the range 1 to 1000 milliseconds.

30 14. An apparatus as claimed in any of claims 5 to 13 wherein the or each outlet port comprises a protruding nozzle.

35 15. A cassette or apparatus as claimed in any of claims 5 to 13 wherein said outlet port comprises an aperture in a wall of the liquid reservoir.

16. A cassette or apparatus as claimed in claim 3 or 15 wherein said aperture has a width of between 2 and 300 micrometers.

17. A cassette comprising a plurality of cartridges for dispensing liquid therefrom, each cartridge comprising an outlet port in the form of a protruding nozzle and a liquid reservoir integrally formed with the outlet port.

5

18. An apparatus or cassette as claimed in any preceding claim wherein the or each liquid reservoir contains a quantity of liquid and is closed at one end thereof by a frangible sealing means.

10

19. A cartridge comprising a liquid reservoir having a predetermined liquid received therein, said reservoir being closed at one end thereof by frangible sealing means, and a nozzle attached to or integrally formed with said reservoir and in fluid communication therewith.

15

20. An apparatus, cartridge or cassette as claimed in claim 18 or 19 wherein said frangible sealing means comprises a foil membrane.

20

21. An apparatus, cartridge or cassette as claimed in any preceding claim wherein said liquid reservoir contains a nucleotide or enzyme.

25

22. An apparatus, cartridge or cassette as claimed in claim 21 wherein said liquid receiving reservoir contains an enzyme selected from a group comprising polymerase, luciferase, adenosine triphosphate (ATP) sulfurylase, and a nucleotide-degrading enzyme such as apyrase.

30

23. A liquid dispensing apparatus including a cassette or cartridge as claimed in any of claims 1 to 4 or 15 to 22.

35

1 / 2

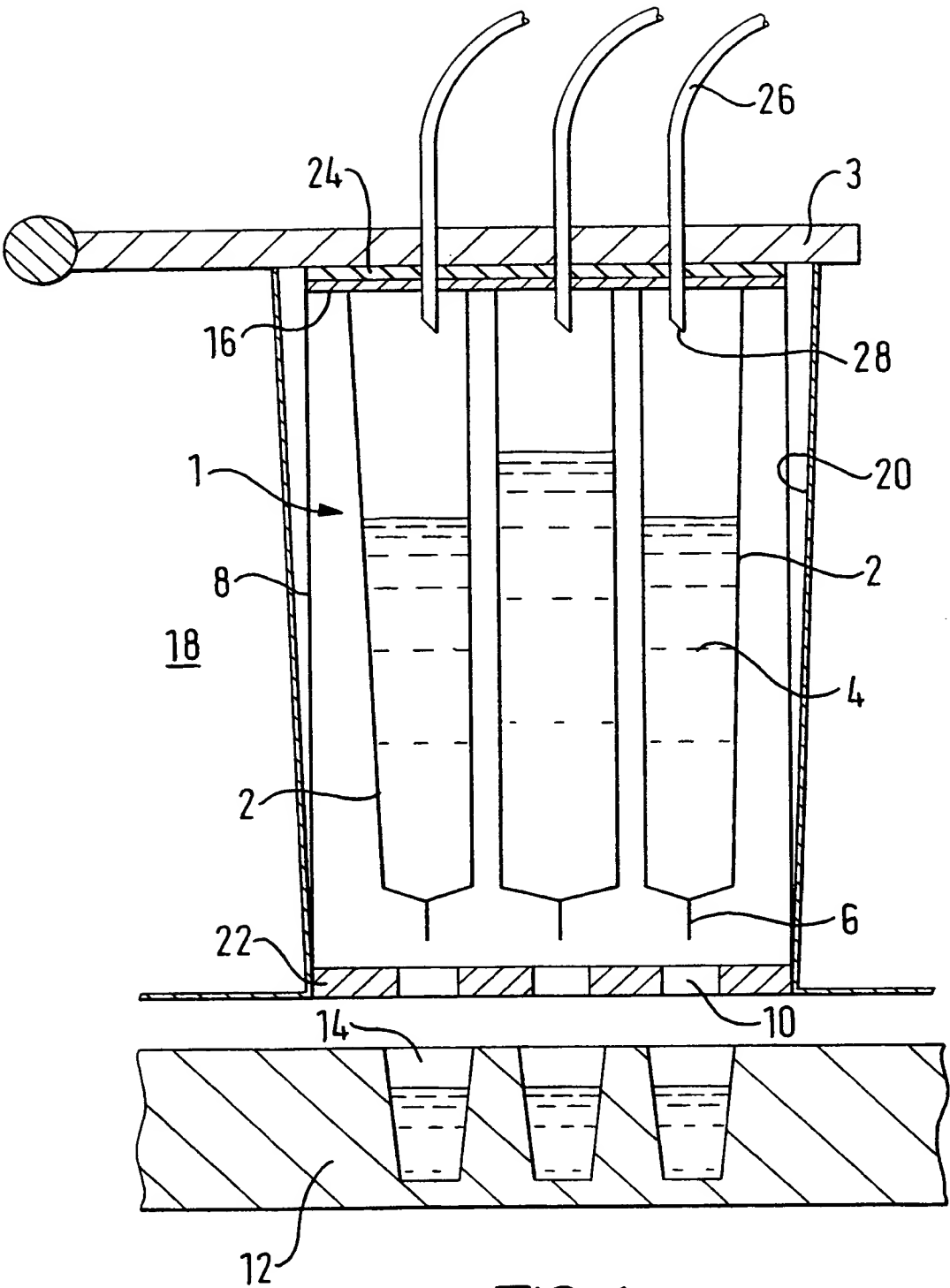


FIG. 1

2/2

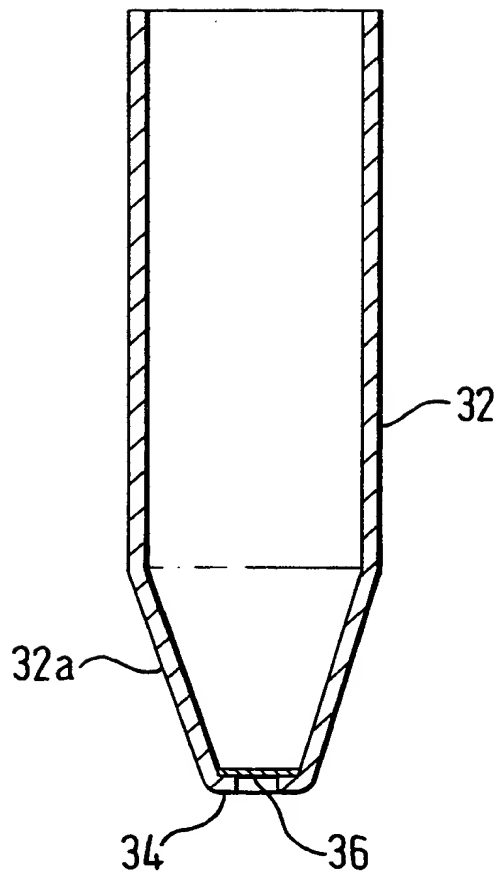


FIG. 2

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 88.96,68343/001	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 01029	International filing date (day/month/year) 20/03/2000	(Earliest) Priority Date (day/month/year) 19/03/1999
Applicant PYROSEQUENCING AB et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

PRESSURE-PULSE ACTUATED LIQUID DISPENSING APPARATUS

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.

1



None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/02

//B01J19/00,C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L G01N B01J C12Q C12M B05C B67D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 435 462 A (FUJII HIDEYO) 25 July 1995 (1995-07-25) column 1, line 46 -column 4, line 4 figure 1	1-3,19, 20,23 17,18
A	---	
A	WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14) page 13, line 15 -page 13, line 18 page 51, line 1 -page 51, line 12 page 52, line 12 -page 54, line 11 figure 2	1-4,17, 23

	-/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

8 June 2000

Date of mailing of the international search report

11 8. 08. 00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Koch, A

PCT/CR 00/01029

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet(s)

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 17-20, 23

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4,17-20,23

Cassette with plurality of cartridges for dispensing liquid therefrom, the cartridge being closed at one end and having an outlet port from which liquid is ejected by a pulse of pressurised gas.

2. Claims: 5-16,21,22

Liquid dispensing apparatus with liquid reservoir, outlet port and driving means for generating a pulse of gas in order to force the liquid out of the outlet port.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/01029

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5435462	A	25-07-1995	NONE	
WO 9820020	A	14-05-1998	US 6024925 A	15-02-2000
			AU 5106998 A	29-05-1998
			AU 5198098 A	29-05-1998
			DE 19782095 T	23-03-2000
			DE 19782096 T	23-03-2000
			EP 0954612 A	10-11-1999
			EP 0937096 A	25-08-1999
			NO 992168 A	06-07-1999
			NO 992169 A	06-07-1999
			WO 9820166 A	14-05-1998
			AU 5247298 A	29-05-1998
			DE 19782097 T	14-10-1999
			EP 0937097 A	25-08-1999
			NO 992167 A	05-07-1999
			WO 9820019 A	14-05-1998
WO 9829736	A	09-07-1998	AU 6646398 A	31-07-1998
			EP 0990142 A	05-04-2000